

**Chemical and biological effects of some mixtures of plant oils and *Thymus vulgaris* on liver diseases**

**Samah M. Ismael<sup>1</sup>, Abour M. Abdelrahman<sup>1</sup>, Eman A. Sultan<sup>2</sup> and Shaimaa M. Omran<sup>1</sup>**

1- Home Economic Dept., Faculty of Specific Education, Ain Shams Univ., Cairo, Egypt

2- Endocrinology and Metabolism Department, National Nutrition Institute, Egypt

**Received: August 3, 2020; Accepted: September 12 2020; Available Online November 25, 2020**

**ABSTRACT**

Liver is the vital organ with a wide range of functions that can influence other body organs. Dietary components are essential for the healthy or diseased liver. Selected food plants can provide nutritional and medicinal support for liver diseases. Herbal and oil medicinal products are increasingly being used and many of them have shown promising potential for the treatment of liver diseases. The aim of this study is to investigate the protective role of thyme, olive oil and flaxseed oil on carbon tetrachloride CCl<sub>4</sub> induced changes in liver enzymes of albino rats.

Sixty adult male Albino rats weighing about 130±5 g were taken and divided into twelve groups, each with five rats. The first group is the control (-) and fed on normal diet for 10 weeks. The second group received subcutaneous injection with CCl<sub>4</sub> in paraffin oil (50% v/v 2 ml/kg) twice per week for 2 weeks to induce chronic damage in the liver tissue and fed on normal diet (control +). Another experimental groups (n = 5/group) were fed a Commercial diet with different doses from thyme, flaxseed oil and olive oil for 10 weeks.

There were a significant increase in the activities of serum ALT and AST in rats of the positive control group (+) as compared to negative control group (-) (P ≤ 0.05). While the protected groups with high doses of thyme (4,6,8,10,12) and olive oil and flaxseed oil (3,5,7,9,11) recorded a significant decreased serum AST and ALT enzyme compared to the low doses groups.

**Recommendations and Conclusion:** High doses of thyme, olive oil and flaxseed oil could ameliorate carbon tetrachloride (CCl<sub>4</sub>)-induced liver injury in rats, which. Suggesting that diet rich in flaxseed oil, olive oil and thyme might be a promising approach for prevention of liver diseases.

**Keyword:** Thyme, Olive oil, Flaxseed oil, CCl<sub>4</sub>, Albino rats, Liver enzymes, Kidney functions, Lipid Profile.

**INTRODUCTION**

Liver plays an important role in vital process of body including metabolism of fat, carbohydrates, and proteins, filtration of microbes, viruses, endotoxins, and antigens, storage of glycogen, vitamins, and minerals, synthesis of clotting factors and albumin, secretion of bilirubin and detoxification of drugs and hormones (Gan *et al.*, 2011). Excess dietary fat and increased insulin glucose level will increase fatty acids in

liver and triglycerides leading to steatosis NASH Non alcoholic steatohepatitis (Balkhy *et al.*, 2016). Liver has a remarkable capacity to adapt to injury through tissue repair, where the multifaceted interactions of immune cell subsets regulate this repair process, such that fibrosis and wound healing can be considered as part of the innate immune response to tissue damage (Farooq *et al.*, 2015).

The most frequent form of liver disease is chronic hepatitis which is defined as a hepatic inflammation that lasts more than 6 months. Hepatitis commonly occurs with hepatitis B and C infections, autoimmune hepatitis, alcoholic and non-alcoholic steatohepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, metabolic causes such as hemochromatosis and Wilson's disease (Ivanova *et al.*, 2017).

About 6% of the world population are chronically infected by the hepatitis (Paoulomi *et al.*, 2012). Viral hepatitis caused 1.34 million deaths in 2015 WHO (2018). Recently great interest has been shifted towards the natural products as medical plants and Essential oils as compared to the classical or synthetic products due to their better affordability, acceptability and compatibility with the human physiology and minimal side effects (Lopez *et al.*, 2017).

Plants food is an essential part of the human diet and comprises various compounds which are closely related to liver health. Selected food plants can provide nutritional and medicinal support for liver diseases (Lei *et al.*, 2017). A vast majority of plants are now being used as phytomedicines. Natural remedies from traditional plants are seen as effective and safe alternative treatments for hepatotoxicity. Several studies have shown that hepatoprotective effects are associated with phyto-extracts/ phyto-compounds rich in natural antioxidants (Nayak *et al.*, 2011). Many bioactive compounds and extracts from plants have thus been investigated for hepatoprotective and antioxidant effects against hepatotoxin – induced liver damage (Yousef *et al.*, 2010).

Olive oil is known for its health benefits. Diet patterns with higher intakes of olive oil are associated with a reduced risk of death from all causes (Al Badr, 2016). It decreases the serum triglycerides, normalizes the liver enzyme biomarkers and significantly reduces the fat droplet

accumulation in liver by suppressing the inflammation and restoring the abnormal lipid metabolisms (Wani *et al.*, 2015).

Flaxseed oil (*Linum usitatissimum* L.) has anti-inflammatory activity with a promising functional food ingredient. Flaxseed oil at different replacement levels resulted in significant improved in lipids profile, liver and kidney. *Thymus vulgaris* L use for treating symptoms of bronchitis, whooping cough and catarrh of the upper respiratory tract. Additional therapeutic properties of thyme include antioxidant (Aristatile *et al.*, 2019) and antimicrobial activity, genotoxic, anti-inflammatory, analgesic and antipyretic effects and antidiabetic effects, among others. Several thyme preparations, such as dried herb, liquid extract, elixir, and tincture, are included in different official monographs, and they are commonly added to formulations involving multiple herbal constituents, most often to syrups, but may be also incorporated in tablets, thyme is to aid digestion of fatty foods (Pankaj *et al.*, 2013).The hepatoprotective effects of thyme have been observed in several experimental models of liver injury. The ethanolic and methanolic extracts of thyme have been effective against aflatoxins- and N-nitroso-diethylamine (NDEA)- induced oxidative liver damage. (Abdel-Aziem *et al.*, 2014).Thyme extract and essential oil could ameliorate carbon tetrachloride (CCl<sub>4</sub>) induced liver injury in rats . The protective effects of aqueous extract and essential oil obtained from thyme (Grespan *et al.*, 2014).

## MATERIALS AND METHODS

### Materials

*Thymus vulgaris* (L.) Burm, Family (lamiaceae) was obtained from the from local market in Cairo. Extra virgin Olive oil produced by Wadi food industries company .Flaxseed oil produced by Imtenan Health Shop. Kits for biochemical analysis were purchased from Gamma Trade for Company Pharmaceutical and Chemicals, Dokki, Giza. Carbon

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tetrachloride CCl<sub>4</sub> (a colorless non-flammable liquid, of molecular weight 153: 84 and freshly diluted in paraffin oil (1:1) volume to a final concentration before use) was obtained from Sigma-Aldrich (SPSS, 1998, London, UK).

### Methods

Moisture, protein, lipid, ash and fiber were determined according the method of AOAC (2000). The total carbohydrate contents were tested quantitatively according to (Kostas *et al.*, (2016). Determination of the anti-oxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H<sub>2</sub>O<sub>2</sub> is determined colorimetrically by an enzymatic reaction which evolves the conversion of 3,5-dichloro -2- hydroxy benzene-sulphonate to a colored product (Chen *et al.*, 2010). Fatty acids in thyme, olive oil and flaxseed oil were identified and quantified in examined formulations by GC/MS (Kaur *et al.*, 2017).

### Biological experiment

#### Animal, housing and diets:

Sixty male Albino rats weighing about 170 ±5 g were obtained from the Agricultural Research Center, Giza, Egypt. The animal groups were kept in an atmosphere of filtered, pathogen-free air and water and maintained at a temperature between 20-25°C with a 12 h light/dark cycle and light cycle (8-20 h) and relative humidity of 50%. The animals acclimatized for one week as an adaptation period. The animals were randomly divided into twelve groups of Five rats. each. The first group of rats, the control (-) fed on commercial diet (Table 1) for 10 weeks (total period of experimental). The second groups was subcutaneous injected with CCL<sub>4</sub> in paraffin oil (50% v/v 2 ml/kg) twice per week by subcutaneous injection for 2 weeks to induce chronic

damage in their liver tissue (Jayasekhar *et al.*, 1997) with fed on commercial diet (control +) as seen in Table (1).

-The other groups were injected with CCL<sub>4</sub> in paraffin oil (50% v/v 2 ml/kg) twice per week by subcutaneous injection for 2 weeks.

- The third and fourth groups fed on commercial diet + thyme (10 % and 15% in substitution of fiber).The fifth and sixth groups fed on commercial diet + Olive oil (7 and 14 mg/rat/ daily).

-The seventh and eighth groups fed on Commercial diet + Flaxseed oil (7 and 14 mg/rat/ daily).

The ninth group feds on Commercial diet +Thyme (10 %/rat/daily) & olive oil (7mg/rat/daily).

-The tenth group fed on commercial diet + Thyme (15 %/rat/daily) & olive oil (14 mg/rat/daily).

-The eleventh group fed on commercial diet + Thyme (10 %/rat/daily) & Flaxseed oil (7 mg/rat/daily).

-The twelfth group fed on commercial diet + Thyme (15 %/rat/daily) & Flaxseed oil (14 mg/rat/daily in Table (2)

The following steps by Schermer (1967) were done in rats after ten weeks of treatment in each group.

\* The animals were fasted for 12 h.

\* Blood samples were withdrawn from orbital plexus venous by using fine capillary glass tubes.

\* Blood samples were collected into plain tubes without anticoagulant and allowed to clot.

\* Blood samples were centrifuged at 3000 rpm for 10 min at 4°C, to obtain clear serum.

\* Serum was frozen at -18°C until analyzed.

\* Animals were anesthetized with ether and sacrificed.

\* They were quickly dissected to excise the liver, kidney, spleen and heart.

\* These organs were weighed and then kept until histological investigations.



**Table (1): Composition of commercial diet.**

Ingredients	Percentage %
Protein:[soy flour meal+ sun flower meal + gluten]	21.00
Fat	03.26
Crude fiber	03.29
DL. Methionine	00.40
Vitamins mixed	01.00
Minerals mixed	04.00
Carbohydrates	67.05

**Table (2): Experimental diets.**

Groups	Experimental diets
<b>Frist</b>	Commercial diet (control (-) group)
<b>Second</b>	CCL <sub>4</sub> + Commercial diet (control (+) group)
Third	(CCL <sub>4</sub> + Commercial diet) + (Thyme (10 % in substitution of fiber).
Fourth	(CCL <sub>4</sub> + Commercial diet) + (Thyme (15 % in substitution of fiber).
Fifth	(CCL <sub>4</sub> + Commercial diet) + (Olive oil (7 mg/rat/ daily).
Sixth	(CCL <sub>4</sub> + Commercial diet + Olive oil (14 mg/rat/ daily).
Seventh	(Commercial diet + Flaxseed oil (7 mg/rat/ daily) + CCL <sub>4</sub>
Eighth	(Commercial diet + Flaxseed oil (14 mg/rat/ daily) + CCL <sub>4</sub>
Ninth	(Commercial diet + Thyme (10 %/rat/daily) & olive oil (7mg/rat/daily)) + CCL <sub>4</sub>
Eighth	(Commercial diet + Thyme (15 %/rat/daily) & olive oil (14mg/rat/daily)) + CCL <sub>4</sub>
Tenth	(Commercial diet + Thyme (10 %/rat/daily) & Flaxseed oil (7 mg/rat/daily) + CCL <sub>4</sub>
Twelfth	(Commercial diet + Thyme (15 %/rat/daily) & Flaxseed oil (14 mg/rat/daily) + CCL <sub>4</sub>

### Histopathology Technique

The tissue sample from liver were fixed immediately after dissection in 10% neutral formalin for 24 h, then collected and dehydrated using ascending grades of alcohol, cleaned in xylene and embedded in paraffin wax. Tissues were sectioned at a thickness of 3 micron and stained with hematoxylin and eosin stains (Banchroft *et al.*, 1996). Then examined by the light microscope for detection of any histopathological alteration.

### Biological Determination

Biological evaluation of the different tested diets was carried by determination of food intake (FI), body weight gain% (BWG %) and organs weight/body weight% according to Chapman *et al.* (1959).

$$\text{BWG\%} = \frac{(\text{Final weight} - \text{Initial weight})}{(\text{Initial weight})} \times 100$$

$$\text{Organ weight / body weight \%} = \frac{(\text{Organ weight} / \text{Final weight}) \times 100$$

### Biochemical analysis

Blood samples were withdrawn from orbital plexus venous by using fine capillary glass tubes, placed in centrifuge tubes without anticoagulant and allowed to clot. After the serum prepared by centrifugation (3000 rpm for 15 min), serum samples were analyzed by biodiagnostic kits.

Serum (uric acid, urea nitrogen and creatinine) were measured colorimetrically using spectrophotometer (model DU 4700) adjusted at 510 nm, 550 nm and 510 nm, respectively by (Barham. (1972) and (Fossati *et al.* 1980), respectively.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined colorimetrically using spectrophotometer (model DU 4700) at 505 nm according to the method of (Tietz.,1990). Serum cholesterol and triglycerides concentrations were determined according to Tietz (1990)

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and Vassalt *et al.* (1986) using spectrophotometer (model DU 4700) at 546 nm and 500-550 nm, respectively.

Lipoprotein (HDL-C and LDL-C) was determined by Fossati and Principe (1982) and Watson (1960) using spectrophotometer (model DU 4700).

### Statistical Analysis

The obtained data were exposed to analysis of variance. Duncan's multiple range test at 5% level of significance was used to compare between means. The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System (SAS, 2006).

## RESULTS AND DISCUSSION

### Physicochemical analyses of Thyme, Olive oil and Flaxseed oil

The proximate composition of thyme, olive oil and flaxseed oil are shown in Table (3). The results of thyme revealed that moisture; carbohydrate, protein, fat, fiber and ash were 17.87, 25.85, 5.80, 1.45, 15.18 and 2.93 %. Thyme powder showed an increase in protein, carbohydrate, crude fiber, ash and fat and decrease in moisture. While, the total fat in olive oil and flaxseed oil were 99.66% and 99.58%, respectively. Thyme constituents of minerals were potassium (590%), sodium (7%), calcium (387%), iron (15.6%). Nawal (2011) mentioned that dry thyme was particularly rich in iron. Iron intake and total iron absorption were highest for the rats fed the dry thyme diet. In the present study the composition of thyme powder presents 17.87% water 5.80% protein, 1.45% fat and 2.93% ash.

**Table (3): Chemical constituent of Thyme, Olive oil and Flaxseed oil.**

Constituents Percentage (%)	Treatments g/100g		
	Thyme	Olive oil	Flaxseed oil
Moisture	17.87±0.73 <sup>B</sup>	0	0
Carbohydrate	25.85± 0.19 <sup>C</sup>	0	0
Protein	5.80±0.27 <sup>B</sup>	0	0
Fat	1.45±0.22 <sup>C</sup>	99.66±0.19 <sup>A</sup>	99.58±0.48 <sup>A</sup>
Fibre	15.18±0.73 <sup>B</sup>	0	0
Ash	2.93±0.47 <sup>B</sup>	0	0
pH	5.52±0.19 <sup>A</sup>	0	0

\* Data are presented as means ± SDM (n=3).

A, B, C: Means with different letter among treatments in the same rows are significantly different.

### Antioxidants:

Antioxidants are component of plant foods play an important role in the treatment of diseases. The obtained results of total antioxidant activity DPPH 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were Olive oil 7.23%, flaxseed oil 12.02 % and Thyme 96.31% (Table 4).

Thyme was the highest percentage compared to olive oil and flaxseed oil as shown in Table (4). Antioxidant activity of thyme was reported to be derived mostly

major phenolic active compounds of thyme, were identified and quantified in examined thyme formulations by GC/MS (Kaur *et al.* ,2017). Found that thymol concentrations of 75.52 [µg/ml], respectively. Concentrations of carvacrol were much lower than those of thymol in, and they were 0.36 [µg/ml]. Accumulation of phenolic compounds may be affected by many factors, such as genetic background, pedoclimatic conditions and agronomic practices.

from the presence of phenolic compounds, particularly thymol and carvacrol, as the

**Table (4): Test result of Antioxidant activity DPPH %**

Antioxidant	activity DPPH %		
	Thyme	Olive oil	Flaxseed oil
DPPH %	96.31	7.23	12.02

Recently, there is a growing interest in phenolic compounds, and flavonoids in particular because of their antioxidant capacity and possible benefits in food and pharmaceutical applications and in human health. Plants have been used for treatment of many diseases (Gülçin *et al.*, 2017). Phenolic compounds are the most widely occurring chemicals, which having strong antioxidant properties (Topal *et al.*, 2018).

Paoulomi *et al.* (2012) found that the type and amount of various antioxidants in flaxseed oil exhibit their antioxidant activity due to their redox property. Measurement of antioxidant activity in olive oil was performed by determination of total antioxidant activity, polyphenol and flavonoid. Gan *et al.* (2011) found that the total phenolic concentration can be improved by extracting oil from destined olives. On the contrary, no effect of destining on olive oil

quality, as measured by acidity, peroxide number, spectrophotometric indices, phenolics, and volatiles. As documented by these studies, destining had no influence on the fatty acid and sterol composition of olive oil.

### Biological evaluation of thyme, olive oil and flaxseed oil on experimental rats:

#### General signs in the rats

No rats among groups died during the experimental period (10 weeks) and all rats groups exhibited no abnormal signs throughout the test period

#### Body and organs weight gain of experimental rats fed on different ratios of *Aloe vera* gel

The final body weights (FBW) of rats for different groups are given in Table (4). There were significant differences ( $P \leq 0.05$ ) in the final body weights of rats in the control (-). The same trend was noticed in the BWG in protected group.

**Table (4). Mean body weight gain (g) of rats fed on different ratios of thyme, olive oil and flaxseed oil**

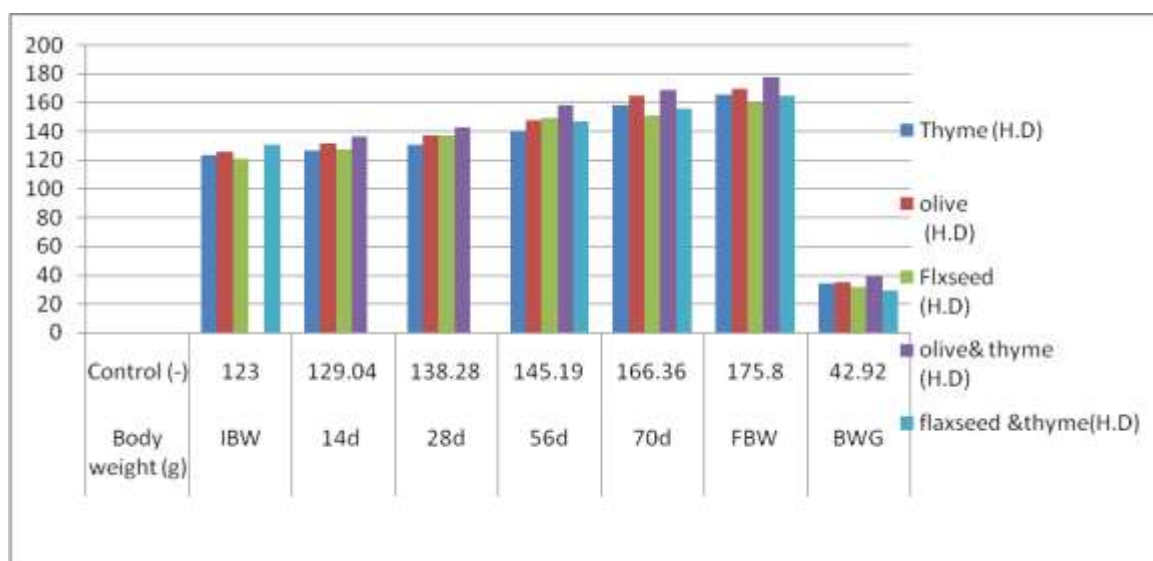
Body weight (g)	Control (-)	Control (+)	Thyme		Olive oil		Flaxseed oil	
			Group (3)	Group (4)	Group (5)	Group (6)	Group (7)	Group (8)
IBW	123.00±2 <sup>a</sup>	125.00±3.1 <sup>a</sup>	124.30±4.0 <sup>a</sup>	123.91±5.8 <sup>a</sup>	123.6±16.8 <sup>c</sup>	126.0±6.2 <sup>b</sup>	126.0±6.2 <sup>b</sup>	121.4±17.8 <sup>c</sup>
14d	129.04±2.4 <sup>ba</sup>	134.33±4.5 <sup>a</sup>	128.51±4.6 <sup>ba</sup>	126.91±5.8 <sup>ba</sup>	128.79±2.7 <sup>b</sup>	131.79±5.3 <sup>a</sup>	133.79±5.34 <sup>a</sup>	127.59±2.7 <sup>b</sup>
28d	138.28±4 <sup>a</sup>	140.54±4.5 <sup>a</sup>	132.98±3.5 <sup>b</sup>	130.63±10.9 <sup>b</sup>	134.31±3.4 <sup>b</sup>	137.27±8.6 <sup>b</sup>	144.71±9.11 <sup>a</sup>	137.67±6.6 <sup>b</sup>
56d	145.19±5.5 <sup>b</sup>	148.06±5.9 <sup>a</sup>	144.85±4.8 <sup>b</sup>	140.38±9.2 <sup>c</sup>	151.70±3.4 <sup>a</sup>	147.88±4.4 <sup>ba</sup>	152.70±3.44 <sup>a</sup>	149.58±3.4 <sup>ba</sup>
70d	166.36±6.2 <sup>a</sup>	151.58±7.7 <sup>c</sup>	162.26±5.1 <sup>b</sup>	158.81±4.4 <sup>b</sup>	158.22±19.7 <sup>b</sup>	164.90±7.49 <sup>ba</sup>	158.22±19.72 <sup>b</sup>	151.58±10.5 <sup>b</sup>
FBW	175.80±22 <sup>b</sup>	155.0±21.7 <sup>c</sup>	176.30±5.3 <sup>b</sup>	166.10±7.1 <sup>ab</sup>	162.30±20.7 <sup>b</sup>	170.00±7.8 <sup>ab</sup>	164.90±7.49 <sup>ba</sup>	160.30±20.7 <sup>b</sup>
BWG	42.92±9.4 <sup>b</sup>	24.0±6 <sup>c</sup>	42.98±4.2 <sup>b</sup>	34.05±4.2 <sup>ab</sup>	36.31±3.6 <sup>bc</sup>	34.92±5.8 <sup>b</sup>	35.72±4.85 <sup>b</sup>	31.31±3.2 <sup>bc</sup>

\* Data are presented as means ± SDM ( $n=8$ ).

Data in a row with different superscript letters are statistically different ( $P \leq 0.05$ )

IBW= Initial body weight; FBW= Final body weight; BWG= Body Weight gain

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**Fig. (1). Body weight gain of rats fed by thyme, olive and flaxseed oil high doses.**

Body weight change is often a very sensitive indicator of animal well being. And it integrates with many other parameters and often, in particular, food consumption. The final body weights (FBW) of rats for different groups are given in Table (4) and Figure (1). There were significant differences ( $P \leq 0.05$ ) in the final body weights of rats in the control (-) ( $175.8 \pm 22.0g$ ) and the remaining treatment. However, there was no

significant difference in animals of group 3 comparing with control (-) group. The lowest rate of body weight gain (BWG) in protected groups (4,6 and 8) and recorded the best result ( $34.05 \pm 4.2$ ,  $92 \pm 5.8$  and  $31.31 \pm 3.2\%$ ) comparing with the control (-) group ( $42.92\%$ ), while values for the remaining treated groups (3,5 and 7) ranged between  $36.31 \pm 3.6\%$  and  $42.98 \pm 4.2\%$ . The lowest rate of body weight gain occurred in control (+) group was  $24.0\%$ .

**Table (5). Mean organs weight (g) body weight % of experimental rats treated by different ratios of thyme, olive oil and flaxseed oil.**

Organs weight(g)	Liver (g)	Kidney(g)	Spleen(g)	Heart(g)
Control (-)	$2.70 \pm 0.33$ b	$0.79 \pm 0.25$ <sup>a</sup>	$0.37 \pm 0.06$ b	$0.32 \pm 0.03$ a
Control (+)	$2.99 \pm 0.09$ a	$0.64 \pm 0.15$ <sup>b</sup>	$0.33 \pm 0.06$ b	$0.30 \pm 0.02$ b
Group 3	$2.85 \pm 0.33$ b	$0.71 \pm 0.08$ <sup>ab</sup>	$0.45 \pm 0.08$ ab	$0.41 \pm 0.09$ b
Group 4	$2.73 \pm 0.23$ b	$0.76 \pm 0.08$ <sup>ab</sup>	$0.41 \pm 0.09$ ab	$0.39 \pm 0.05$ b
Group 5	$2.70 \pm 0.33$ <sup>ab</sup>	$0.79 \pm 0.26$ <sup>a</sup>	$0.48 \pm 0.16$ a	$0.31 \pm 0.03$ a
Group 6	$2.65 \pm 0.29$ ab	$0.78 \pm 0.25$ a	$0.34 \pm 0.1$ ab	$0.30 \pm 0.04$ a
Group 7	$2.99 \pm 0.09$ <sup>a</sup>	$0.64 \pm 0.15$ b	$0.35 \pm 0.04$ ab	$0.31 \pm 0.06$ a
Group 8	$1.95 \pm 0.24$ <sup>b</sup>	$0.65 \pm 0.08$ b	$0.36 \pm 0.2$ ab	$0.31 \pm 0.03$ a
Group 9	$2.31 \pm 0.12$ <sup>a</sup>	$0.70 \pm 0.07$ ab	$0.43 \pm 0.13$ a	$0.38 \pm 0.05$ b
Group 10	$2.65 \pm 0.29$ <sup>ab</sup>	$0.79 \pm 0.26$ <sup>a</sup>	$0.37 \pm 0.06$ b	$0.30 \pm 0.04$ b
Group 11	$2.30 \pm 0.11$ <sup>a</sup>	$0.72 \pm 0.06$ <sup>ab</sup>	$0.38 \pm 0.05$ ab	$0.31 \pm 0.02$ a
Group 12	$1.90 \pm 0.23$ <sup>b</sup>	$0.79 \pm 0.26$ <sup>a</sup>	$0.37 \pm 0.04$ <sup>a</sup>	$0.32 \pm 0.03$ a

\* Data are presented as means  $\pm$  SDM ( $n=8$ ).

Data in a row with different superscript letters are statistically different ( $P \leq 0.05$ )



The weights of various organs/body weight % of rats are shown in Table (5). The weights of the organs (liver, kidney, spleen and heart) of rats maintained on experimental diets (+) thyme powder substitution of fiber 10% (group 3) were (2.85±0.33, 0.71±0.08, 0.45±.08 and 0.41±0.09g), while thyme substitution of fiber for 15% (group 4) were (2.73±0.23, 0.76±0.08, 0.41± 0.09 and 0.39± 0.05g) and olive oil for 7mg in group 5 were (2.70± 0.33, 0.79±0.26, 0.48±0.16 and 0.31± 0.03g), respectively. Flaxseed oil for 7 mg (group 7) were (2.99±0.09, 0.64±0.15, 0.35±0.04 and 0.31± 0.06g), while for 10 mg (group 8) were (1.95±0.24, 0.65±0.08, 0.36±0.2 and 0.31± 0.03g). In injected groups with CCl<sub>4</sub>, there was almost significant difference in the weight of liver, kidney, spleen and heart of rats from control (+) groups. The remaining 3 treatments

(injected group) were either show ratio of weight change or suffered a weight loss in liver comparing with control (+).

### Biochemical analysis

Results of biochemical analysis for all tested groups are presented in Table (6). Alterations in the liver enzyme (ALT and AST) were statistically significant ( $P \leq 0.05$ ) in all tested groups. The results demonstrated that ALT and AST levels in the positive control group (+) recorded a significant increase ( $P \leq 0.05$ ) in the activities of serum ALT and AST (15.37±1.48 and 33.67±3.21 mg/dl) as compared to the negative control group (-) (9.33 ±1.52 and 17.52±2.47mg/dl). While the protected samples (thyme & olive oil and flaxseed oil) showed different ratios of decrease in serum AST and ALT enzyme compared to the control (+) group (Table (6) and Figure (2)).

**Table (6). Liver and kidney function of experimental rats treated by different ratios of Thyme, Olive oil and flaxseed oil.**

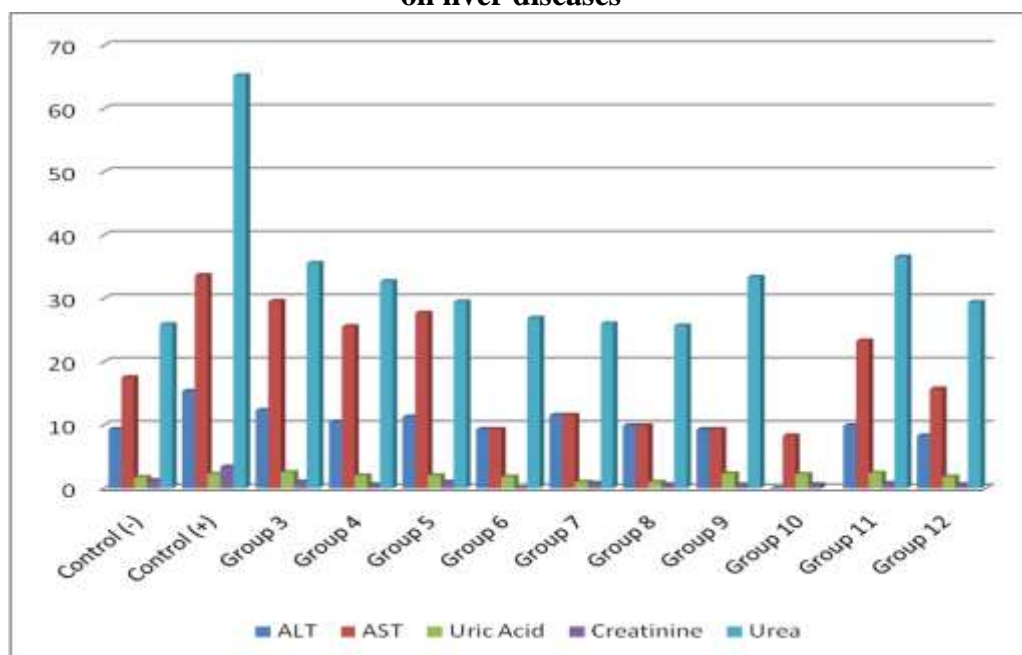
Parameters	ALT (U/L)	AST (U/L)
Control (-)	9.33 ±1.52 <sup>c</sup>	17.52±2.47 <sup>c</sup>
Control (+)	15.37±1.48 <sup>a</sup>	33.67±3.21 <sup>a</sup>
Group 3	12.34±0.55 <sup>a</sup>	29.55±2.79 <sup>a</sup>
Group 4	10.50±0.58 <sup>a</sup>	25.58±4.69 <sup>b</sup>
Group 5	11.30±0.42 <sup>b</sup>	27.70±1.82 <sup>b</sup>
Group 6	9.32 ±1.62 <sup>c</sup>	9.32 ±1.62 <sup>c</sup>
Group 7	11.58±1.68 <sup>a</sup>	11.58±1.68 <sup>a</sup>
Group 8	9.90±0.10 <sup>b</sup>	9.90±0.10 <sup>b</sup>
Group 9	9.32 ±1.62 <sup>c</sup>	9.32 ±1.62 <sup>c</sup>
Group 10	9.02±0.10 <sup>b</sup>	8.32 ±1.62 <sup>c</sup>
Group 11	9.90±0.10 <sup>b</sup>	23.30±2.30 <sup>a</sup>
Group 12	8.32 ±1.62 <sup>c</sup>	15.72±2.37 <sup>c</sup>

\*Data are presented as means ± SDM ( $n=8$ ).

Data in a row with different superscript letters are statistically different ( $P \leq 0.05$ ).

AST: aspartate amino transferase, ALT: alanine amino transferase

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**Fig. (2). Effect of different ratios of Thyme, Olive oil and flaxseed oil on Liver and Kidney function of experimental rats**

The serum levels of ALT, AST, and GGT reflect the physiological state of the liver; they are changed according to the distortion of liver, resulting from cellular injury of the organ caused by toxic metabolites and diseases (Girish *et al.*, 2019). Results of Parmar *et al.* (2010) indicated that CCl<sub>4</sub> caused an increase in serum levels of the diagnostic enzymes (ALT, AST and GGT) in rats that received CCl<sub>4</sub> as compared to the control group.

Ozkol *et al.* (2015) found that CCl<sub>4</sub> induced liver damage, there is an excessive lipid peroxidation leading to functional and structural disruption. The damage or death of hepatocytes usually results in the leakage of the enzymes in the affected tissue into the blood stream (Lee *et al.*, 2018).

Some bioactive compounds of thyme are very effective such as tinnins, steroids and alkaloids. Specific steroids and flavonoids are responsible to protect the liver from oxidative stress and play a key role in hepatoprotection (Joseph, and Raj, 2010). Olive oil possesses hepatoprotective activity and reduces the level of ALT (Nayak *et al.*, 2011)

The present results demonstrated that ALT and AST levels were significantly lower in groups injected with CCl<sub>4</sub> compared with control (+) group. Increased serum creatinine above normal levels may reflect destroy of 50% of renal nephron (Girish *et al.*, 2019).

#### **Lipid profile of rats fed on different ratios of thyme, olive oil and flaxseed oil.**

Results of lipid profile analysis for all tested groups are presented in Table (7) and Figure (3). The results demonstrated significant increase ( $p \leq 0.05$ ) in the values of cholesterol, triglycerides, HDL-c and LDL-c in control positive group comparing with other treatments in protected and injected groups. It was obvious from Table (7) and Figure (3) that the highest decrease in lipid profile and triglycerides was in groups 4,6 and 8 which recorded  $91.60 \pm 15.57$ ,  $88.90 \pm 38.08$  and  $92.60 \pm 38.08$  mg/dl comparing with control (+)  $99.50 \pm 14.70$  mg/dl in protected group. This result agreed with Rota *et al.* (2017) who found that Triglycerides concentrations were significantly decreased in serum of CCl<sub>4</sub> treated

animals. CCl<sub>4</sub> induced a slight decrease in cholesterol levels, which was partially recovered after administration of thyme tincture. The intake of thyme preparations alone did not affect significantly the metabolic function of the liver. Impaired excretory function of the liver was observed after treatment of animals with CCl<sub>4</sub>. The result in Table (7) showed that Cholesterol recorded high concentration in groups 4,6 and 8 ( 84.50±5.51, 79.60±7.05 and 85.60±7.05 mg/dl, respectively) comparing with control (+) (89.70±13.29

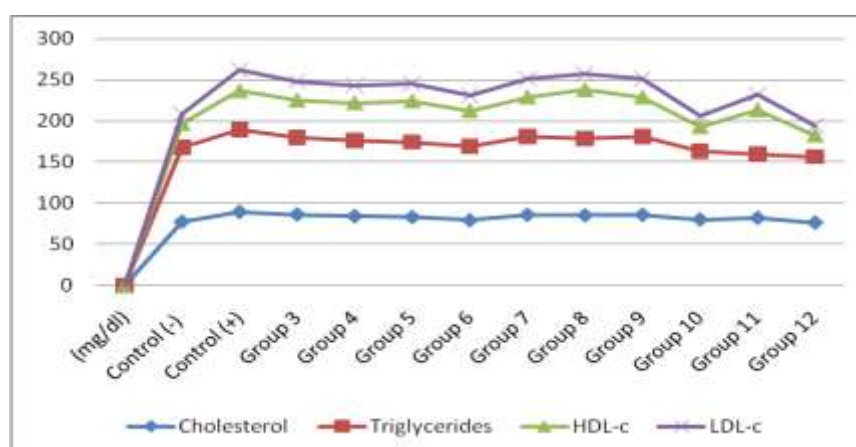
mg/dl). LDL-c levels in groups (4,6 and 8) were 20.76±5.86, 18.48±5.69 and 18.48±9.79 mg/dl, respectively comparing with control (+) group (25.09±12.36 mg/dl). Farooq *et al.* (2015) showed that the supplementation of olive oil decreases the serum triglycerides, normalizes the liver enzyme biomarkers and significantly reduces the fat droplet accumulation in liver by suppressing the inflammation and restoring the abnormal lipid metabolisms in experimental animals.

**Table (7). Lipid profile of experimental rats treated by different ratios of Thyme, Olive oil and flaxseed oil.**

lipid profile (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
Control (-)	77.90±19.44 <sup>cb</sup>	89.40±7.40 <sup>c</sup>	29.80±6.03 <sup>c</sup>	10.82±15.75 <sup>c</sup>
Control (+)	89.70±13.29 <sup>a</sup>	99.50±14.70 <sup>a</sup>	47.50±9.65 <sup>ab</sup>	25.09±12.36 <sup>a</sup>
Group 3	86.60±13.93 <sup>b</sup>	92.90±9.81 <sup>b</sup>	45.80±3.91 <sup>b</sup>	22.64±12.07 <sup>a</sup>
Group 4	84.50±5.51 <sup>ab</sup>	91.60±15.57 <sup>b</sup>	46.00±3.63 <sup>b</sup>	20.76±5.86 <sup>b</sup>
Group 5	83.50±12.28 <sup>c</sup>	90.50±11.31 <sup>ab</sup>	50.50±17.87 <sup>a</sup>	20.28±10.64 <sup>ab</sup>
Group 6	79.60±7.05 <sup>b</sup>	88.90±38.08 <sup>ab</sup>	44.00±13.86 <sup>a</sup>	18.48±5.69 <sup>b</sup>
Group 7	86.20±10.29 <sup>a</sup>	94.40±4.34 <sup>b</sup>	48.60±3.21 <sup>b</sup>	21.56±3.32 <sup>b</sup>
Group 8	85.60±7.05 <sup>b</sup>	92.60±38.08 <sup>ab</sup>	60.00±12.86 <sup>a</sup>	18.48±9.79 <sup>b</sup>
Group 9	86.20±10.29 <sup>a</sup>	94.40±4.34 <sup>b</sup>	48.60±3.21 <sup>b</sup>	21.56±3.32 <sup>b</sup>
Group 10	80.60±7.05 <sup>b</sup>	82.60±38.08 <sup>ab</sup>	30.00±12.86 <sup>a</sup>	12.48±9.79 <sup>b</sup>
Group 11	82.40±11.28 <sup>c</sup>	76.80±14.34 <sup>cb</sup>	54.40±16.97 <sup>a</sup>	18.28±10.64 <sup>ab</sup>
Group 12	76.80±14.34 <sup>cb</sup>	79.40±7.50 <sup>c</sup>	26.60±5.03 <sup>c</sup>	10.72±15.75 <sup>c</sup>

\* Data are presented as means ± SDM (n=6).

Data in a row with different superscript letters are statistically different ( $P \leq 0.05$ ).



**Fig. (3). Effect of different ratios of Thyme, Olive oil and flaxseed oil on lipid Profile of experimental rat**

Daun and DeClercq (1994) found that flaxseed oil lowered the total cholesterol by 61% and increased the

proportion in the high density lipoprotein (HDL). Diederichsen and Richards (2015) reported that pre-treatment of rats with

flaxseed oil significantly reduced the CCl<sub>4</sub> induced lipid peroxidation in liver and biochemical changes associated with CCl<sub>4</sub>. Rašković *et al.* (2015) evaluated antioxidant activity of thyme preparations and their influence on hepatic function using the hepatotoxicity model induced by CCl<sub>4</sub>. The pharmaceutical formulations containing thyme may aggravate existing hepatotoxicity. On the contrary, the hepato-protective effects of thyme have been observed in several experimental models of liver injury. The ethanolic and methanolic extracts of thyme have been effective against aflatoxins- and N-nitrosodiethylamine (NDEA)-induced oxidative liver damage (Abdel-Aziem *et al.*, 2014; Noor *et al.*, 2015).

### Histopathological examination

Organs such as liver were examined by a histological approach and the photomicrographs of hematoxylin – eosin stained liver is illustrated.

### Liver

The liver was examined by a histological approach and the photomicrographs of hematoxylin. The liver sections from control (-) group (normal rats fed on commercial diet only) showed normal histological structure of the central vein and surrounding hepatocytes in the hepatic parenchyma. There was no histopathological alteration and the normal histological structure of the central vein and surrounding hepatocytes in the parenchyma were recorded in (Fig.4.1). While in control (+) group of experimentally induced rats by administration of CCl<sub>4</sub>. Focal lipidosis was observed in the hepatic capsule as well as the underlying hepatocytes in the parenchyma associated with necrosis and degenerative changes in the hepatocytes underneath (Fig.4.2)

Animals fed on thyme 10% in substitution of fiber, group of experimentally induced rats and treated by low dose of thyme. The hepatic capsule

showed focal lipidosis associated with atrophy in the underlying hepatocytes in the parenchyma were recorded (Fig.4.3) There were oedema and few inflammatory cells infiltration with congestion in the portal vein at the portal area (Fig.4-12&13). Rats fed on thyme 15% in substitution of fiber, group of experimentally induced rats and treated by 15 % dose of thyme. The portal area showed dilatation in the portal vein as well as periductal inflammatory cells infiltration surrounding the bile ducts (Fig.4.4). Group of experimentally induced rats and treated by 7mg dose of olive oil. Sever congestion was observed in the central vein and sinusoids associated with focal hemorrhage in the hepatic parenchyma (Fig.4.5). While group of experimentally induced rats and treated by 14mg dose of olive oil showing Few inflammatory cells infiltration was detected in the portal area (Fig.4.6)

Group of experimentally induced rats and treated by 7 mg dose of flaxseed oil The portal area showed few inflammatory cells infiltration (Fig.4.7). While group of experimentally induced rats and treated by 14 mg dose of flaxseed oil .There was no histopathological alteration as recorded in (Fig.4.8). Rats fed on 7 mg dose of olive oil and 10% thyme .The portal area showed few inflammatory cells infiltration (Fig.9). Group of experimentally induced rats and treated by 14 mg dose of olive oil and 15% thyme. There was no histopathological alteration as recorded in (Fig.4.10). Group of experimentally induced rats and treated by 7 mg dose of Flaxseed oil and 10% thyme. Dilatation was observed in the central and portal veins associated with oedema and few inflammatory cells infiltration in the portal area (Fig. 4.11). Group of experimentally induced rats and treated by 14 mg dose of Flaxseed oil and 15% thyme. There was no histopathological alteration as recorded in (Fig.4.12).

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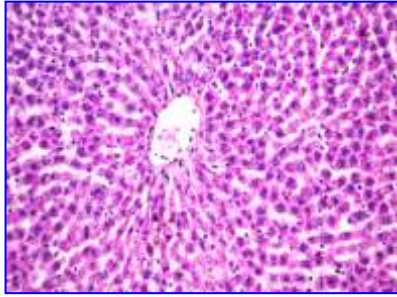


Fig. 4-1 Control (-)

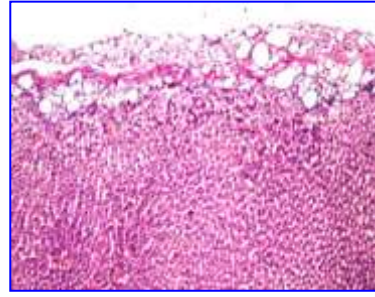


Fig. 4-2 Control (+)

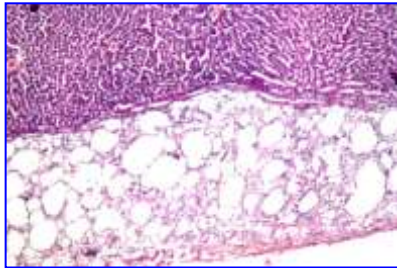


Fig. 4-3.

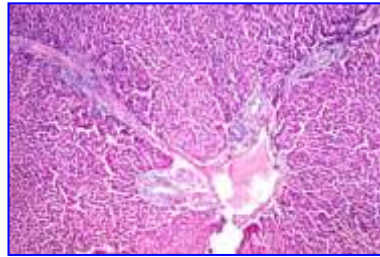


Fig. 4-4

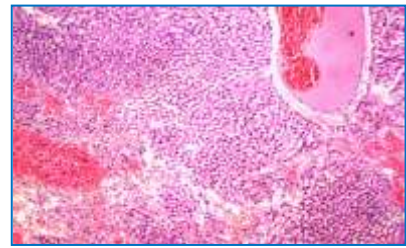


Fig. 4-5

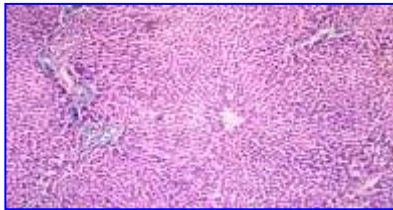


Fig. 4-6

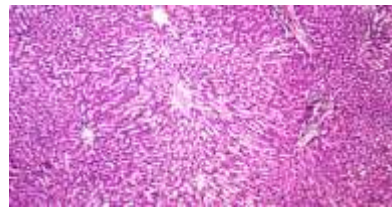


Fig. 4-7

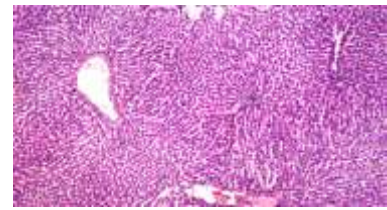


Fig. 4-8

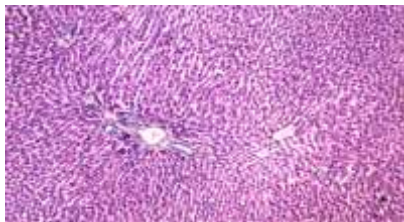


Fig. 4-9

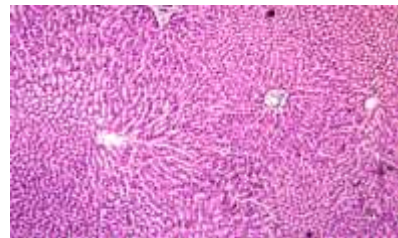


Fig. 4-10

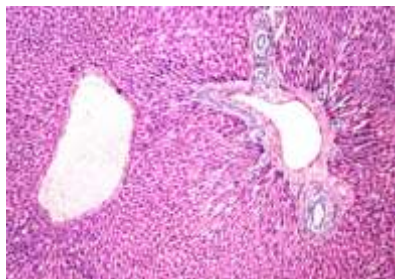


Fig. 4-11

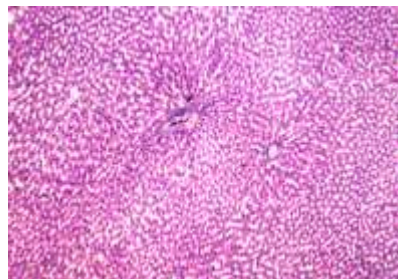


Fig. 4-12

Fig (4): Sections of liver showing histological changes stained with H&E on using different doses of Thyme, Olive oil and flaxseed oil in protected and injected groups.

**Legend of Fig. 4.**

- Fig. 4-1** Showing normal histological structure of the central vein and surrounding hepatocytes. X40
- Fig. 4-2** Showing Focal lipidosis in the capsule as well as with degenerative and necrosis of hepatocytes. X 20
- Fig. 4.3** Showing Focal lipidosis in the hepatic capsule with atrophy in the underlying hepatocytes (group 3). X 20
- Fig. 4.4** Showing dilatation in the portal vein with few periductal inflammatory cells infiltration surrounding the bile ducts at the portal area (group 4). X 20
- Fig. 4.5** Showing severe congestion in the central vein and sinusoids with focal hemorrhage in the hepatic cells (group 5). X 20
- Fig. 4-6** Showing few inflammatory cells infiltration in the portal area (group 6). X 20
- Fig. 4-7** Showing few inflammatory cells infiltration in portal area (group 7). X 20
- Fig. 4-8** Showing normal histological structure (group 8). X 20
- Fig. 4-9** Showing few inflammatory infiltration cells in portal area (group 9). X 20
- Fig. 4-10** Showing normal histological structure (group 10). X 20
- Fig. 4-11** Showing dilatation central and portal vein with oedema and few inflammatory cells infiltration in portal area (group 11). X 20
- Fig. 4-12** Showing normal histological structure (group 12). X 20

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التأثيرات الكيميائية والبيولوجية لخليط من الزيوت النباتية والزعر البري على أمراض الكبد

سماح محمد إسماعيل<sup>1</sup> ، عبور عبدالرحمن<sup>1</sup> ، إيمان سلطان<sup>1</sup> ، الشيماء محمد عمران<sup>1</sup>  
 1- قسم الإقتصاد المنزلي -شعبة التغذية وعلوم الأغذية- كلية التربية النوعية - جامعة عين شمس - القاهرة- مصر  
 2- قسم الغدد الصماء والتمثيل الغذائي ، المعهد القومي للتغذية

### المستخلص

المنتجات الطبية العشبية يتم استخدامها بشكل متزايد ، وقد أظهر العديد منها إمكانات واعدة لعلاج أمراض مختلفة. معظم الآثار الدوائية للزعر هي نتيجة النشاط المضاد للأكسدة العالي الذي يعزى بشكل رئيسي إلى وجود الفينول ، الثيمول والكرفاكول ، المكونات الرئيسية لزيت الزعر الأساسي. الأحماض الدهنية غير المشبعة طويلة السلسلة (LCPUFA) التي يتم تصنيعها بشكل رئيسي في الكبد لها وظائف ذات صلة في الكائنات الحية إن المستويات المنخفضة من الأحماض الدهنية غير المشبعة المتعددة (PUFAs) في خزعات المصل والأنسجة الكبدية هي الخصائص الشائعة في المرضى الذين يعانون من أمراض الكبد حمض  $\alpha$ -linolenic (ALA) هو مستخرج من نبات n-3 PUFA و غني بزيت بذور الكتان وزيت الزيتون ، وقد أجريت هذه الدراسة للتحقق من الدور الوقائي للزعر وزيت الزيتون وزيت بذور الكتان على رابع كلوريد الكربون تسبب CCl<sub>4</sub> التغيرات في إنزيمات الكبد في ال فئران البيضاء. تم أخذ ستين فأر من الذكور البالغ وزنها حوالي  $130 \pm 5$  جرام وتقسيمها إلى اثني عشر مجموعة ، كل مجموعة تحتوي خمس فئران. المجموعة الأولى هي المجموعة الضابطة (-) وتتغذى على نظام غذائي عادي لمدة 10 أسابيع. تلقت المجموعة الثانية الحقن تحت الجلد مع CCl<sub>4</sub> في زيت البرافين ( 50% v / v 2 ml / kg ) مرتين أسبوعياً لمدة أسبوعين لإحداث تلف مزمن في أنسجة الكبد وتغذيت على نظام غذائي عادي ( + control ). مجموعات تجريبية أخرى ( ن = 10 / مجموعة) تم تغذيتها بنظام غذائي تجاري بجرعات مختلفة من الزعر وزيت الكتان وزيت الزيتون لمدة 10 أسابيع. أظهرت النتائج أن مستويات ALT و AST كانت مجموعة الضبط الإيجابية (+) سجلت زيادة معنوية (  $P \leq 0.05$  ) في أنشطة مصل ALT و AST مقارنة بمجموعة الضبط السلبية (-). بينما المجموعات المحمية بجرعات عالية من (الزعر وزيت الزيتون وزيت بذور الكتان) سجلت انخفاضا كبيرا في مصل إنزيم ALT و AST مقارنة بمجموعة الجرعات المنخفضة ، كما انخفضت القيم المتوسطة لحمض اليوريك واليوريا والكرياتينين في جميع المجموعات المختبرة مقارنة بالمجموعة الضابطة (+ مجموعة النتائج: أظهرت النتائج أن الجرعات العالية من الزعر وزيت الزيتون وزيت بذور الكتان يمكن أن تخفف من إصابات الكبد التي يسببها رابع كلوريد الكربون ( CCl<sub>4</sub> ) في الفئران ، والتي وجدت أن تركيزات مستويات الدهون الثلاثية والكوليسترول انخفضت بشكل كبير في مصل الحيوانات المعالجة. مما يشير إلى أن النظام الغذائي الغني بزيت بذور الكتان وزيت الزيتون والزعر قد يكون نهجاً واعداً للوقاية من أمراض الكبد. كما أظهرت نتائج الوصف التشريحي حدوث تحسن في تغيرات نسيج الكبد والكلى للفئران التي تم أصابتهم برابع كلوريد الكربون والتي تغذت على نسب عالية من الزعر وزيت الزيتون وزيت بذور الكتان في المجموعات الوقائية.